# IDENTIFICATION, ISOLATION, AND CHARACTERIZATION OF ACTIVE PRINCIPLES FROM *Euphorbia royleana*

**SYNOPSIS**

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## Introduction

Since ancient times humanity is directly or indirectly dependent on plants. As they provide us medicines, shelter, food, etc. The knowledge of folk traditions of medicine had been transferred from our ancestors to us. (Kargiouglu et al., 2008). Medicinal plants not only play an important role in India in the medical field but also in the socio-cultural and spiritual area (Dafni, 2006). Moreover, the increasing dependence on the use of medicinal plants in industrialized societies has been traced to the extraction and improvement of many medicines and chemical treatments from these plants as well as from rural herbal remedies which are used traditionally. Also, the worlds health Organisation (WHO) has cataloged that 80% of the world's population in a developing country uses traditional medicines (use of plants) (Sasidharan et al., 2011). The family Euphorbiaceae has a wide range of diversity and comprises large woody trees to simple weeds (Mwine & Damme, 2011). Euphorbiaceae is one of the biggest families of flowering plants, composed of over 300 genera and 8000 species (Webster, 1994; Kemboi, 2020). There are over 2000 species in the genus *Euphorbia* and is one of the largest genera in the family Euphorbiaceae (Vasas & Hohmann, 2014). The genus can be found in all temperate and tropical regions and has a worldwide distribution (Salehi, 2019). Due to wide diversity, various secondary metabolites with bioactivities are present in the species of genus *Euphorbia* and so the diversity in ethnomedicine (Seigler, 1994; Shi, Su & Kiyota, 2008). Plant bioactive components comprise a variety of compounds (tannins, lignans, coumarins, quinones, stilbenes, phenolic acids, flavones, flavanols, etc.) that may help to prevent the onset of degenerative diseases and extend life hope (Robards et al., 1999). Several plants of genus euphorbia are known as folk herbal medicine mainly in 'Chinese traditional medicine' (Kumar, Malhotra & Kumar, 2010). *Euphorbia* species are widely used for the treatment of, ascites, cancer (Ernst et al., 2015). In Chinese and Ayurveda medicine, *Euphorbia* species were used for the treatment of liver diseases, snake bites, respiratory disorders, asthma, and rheumatism (Kemboi et al., 2020). Usable parts of *Euphorbia* species for medicine include latex, wood, bark, leaves, roots, seeds, and whole plants (Pascal et al., 2017). For this reason, the pharmaceutical industry relies on these systems for the production of bio-active ingredients from medicinal plants, and researchers from the biotechnology and chemistry field take it as a scope of interest for the research (Mustafa et al., 2017).

The reason behind the demand and use of medicinal plants is the availability of natural products in the plant parts which are less toxic and do not show side effects for the treatment of disease. Chemicals produced by living beings are natural products. Valuable contents (natural products) present in plants act as an antioxidant and some of them are used as food for humans (Boots, Haenen, & Bast, 2008). Earlier reports say that the plants contain vitamins, flavonoids, phenolics and are considered as a primary source of antioxidants (Suffredini et al., 2004). Natural products play an important role in the treatment of cancer and infectious diseases (Atanasov et al., 2015; Harvey, Edrada-Ebel & Quinn, 2015). Millions of years of evolution have conferred natural products with a unique chemical diversity, which is reflected in the wide variety of their biological activities and the properties that make them suitable for drug development. Therefore, even before modern chemical pharmacology was introduced, NPs were used for centuries as active ingredients in traditional medicines, especially in herbal remedies (Sorokina & Steinbeck, 2020). In recent years, natural products have been attracting the attention of the scientific community, and their interest is steadily increasing. As a result, over the last two decades, various databases and collections have grown rapidly as a general resource for natural products information (Sorokina & Steinbeck, 2020). In the cosmetic industry (Dunkel et al., 2009), food industry (Yue et al., 2014; Ahmed et al., 2011), and development in the agriculture field with pesticides (Sparks et al., 2019), the natural products and its derivatives are thoroughly studied and use. In the past, natural products (NPs) have played an important role in drug discovery, especially in infectious diseases and cancer therapy (Atanasov et al., 2015; Harvey, Edrada-Ebel & Quinn, 2015). Also, in other therapeutic areas such as cardiovascular disease and multiple sclerosis (Harvey, Edrada-Ebel & Quinn, 2015; Newman & Cragg, 2016; Waltenberger et al., 2016). Various phytochemicals such as flavonoids, alkaloids, phenolic acids, anthocyanins, lignans, stilbenes, polysaccharides, carotenoids, essential oils, and certain vitamins (A, C, E) can prevent and treat dangerous things like diseases such as cancer, diabetes, Alzheimer's disease, cardiovascular disease and hence natural products are getting more and more attention because of its important role (Xiao, Muzashvili & Georgieve, 2014; Andrae-Marobela, 2013).

Different species of the *Euphorbia* genus of the Euphorbiaceae family are traditionally used for the treatment of skin problems (Kichu, 2015), snake bites (Kumar & Choyal, 2012), joint and leg pain (Burlakoti & Kunwar, 2008). Euphorbiaceae is scientifically

reported for its antiviral (Zheng, Cui, & Zhu, 1998), antimicrobial (Zheng, Cui & Zhu, 1998), anticancer, cytotoxic, and antitumor activities (Zhang, 2017). Genus *Euphorbia* is known as spurges and is commonly used as an ornamental plant (Pascal et al., 2017). On cutting, spurges usually exude white latex (Pascal et al., 2017; Kumar, Malhotra, & Kumar, 2010). Genus *Euphorbia* consists of many diterpenoids and till now more than 700 diterpenoids have been isolated (Vasas & Hohmann, 2014) *Euphorbia royleana* is locally known as sullu, chuuein (chuu), thor, etc. around the north-western ghats of India (Chaitanya & Suresh 2018; Thakur & Sood, 2001). It is a thorny succulent shrub and gives an appearance like cactus (Shakera et al., 2020; Wang et al., 2019; Paudel, et. al., 2021). It is a perennial plant, and the false flowers are almost stalkless. Leaves are green and fleshy and are arranged alternatively. Plant leaves get shredded off in the hot and summer season (Wu, Raven & Hong, 1862). Stems are thick and sponges, 5-7 angled. Prickles are attached to it and are paired. *Euphorbia royleana* communities are found in dry rocky hill slopes of eastern and western ghats of the Himalayas (Bani et al., 2000; Bani et al., 2005). The plant is used traditionally for the treatment of many ailments like paralysis, loose motions, bladder stones, and ear pain (Sabeen & Ahmad, 2009). Piscicidal and anti-acetylcholinesterase activity are shown by the Stem bark extract of *Euphorbia royleana* (Tiwari & Singh, 2004). Anti-inflammatory and anti-arthritic activities are shown by the latex of the plant (Bani et al., 2000). And, it is the root of the plant that is highly utilized as an ethnomedicine since it contains a high concentration of bioactive compounds.

The scientific classification of the plant *Euphorbia royleana* is: -

Kingdom - Plantae

Division - Tracheophyta

Class – Spermatophyta

Order – Magnoliopsida

Family – Euphorbiaceae

Genus – *Euphorbia*

Species – *royleana*

Fig. 1. *Euphorbia royleana* plant

Fig. 2. Roots of *Euphorbia royleana*

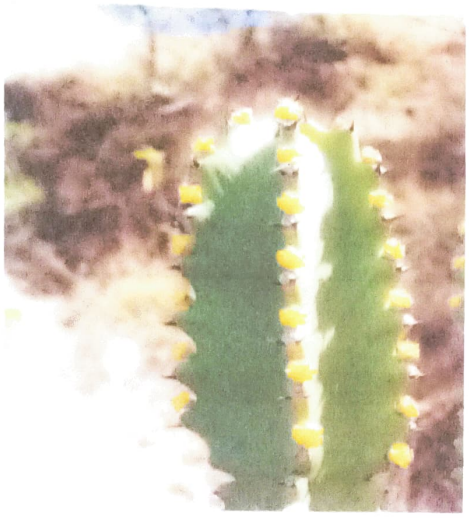


Fig. 3. *Euphorbia royleana* flower

Fig. 4. *Euphorbia royleana* leaves

So, by looking at the strong medicinal properties of plants, *Euphorbia royleana* is selected for further research.

## Literature Review

Nadkarni & Nadkarni (1982) demonstrate the medicinal plants of India in the book "The Indian Materia Medica". Uses of genus Euphorbia are also mentioned in the book. The *Euphorbia royleana* is a medicinal plant and shows anthelmintic and cathartic properties. Rizk et al. (1984) The resin pronounced pro-inflammatory activity on mammalian skin which was prepared by extraction and partition of *Euphorbia royleana* latex, which is an ether soluble. The inactive fraction is separated from the biologically active fraction by dry column chromatography further separated by adsorption and partition thin-layer

methods. The least polar zone consisted of an inseparable mixture of four tetra-esters of the macrocyclic, diterpene, ingol, whilst the more polar fraction consisted of a mixture of four in-esters of the same diterpene.

Rizk (1987) made the review of chemicals on family Euphorbiaceae of a different class of compounds that have been isolated from the plants. And, economical uses of members of the *Euphorbia* family. Plant *Euphorbia royleana* contains 3 benzocoumarin and 1 phenolic acid (7-hydroxy-3,4-benzocoumarin, 7-methoxy-3,4-benzocoumarin, 2,7-dihydroxy,3-4-benzocoumarin, and m-hydroxybenzoic acid) respectively.

The study conducted by Ogbulie et al. (2007) revealed that ethanolic extracts of *E. hirta* leaves showed higher activity against the gram-positive bacteria S. aureus (MIC = 22.55 mg/mL) than against the other microorganisms tested. This is due to the presence of tannins, alkaloids, and flavonoids. But it was much less active than the ethanol extract of aerial parts against the same S. aureus strain (MIC= 1.95 µg/mL. And thus, this justifies the use of the plant in earlier times for the treatment of boils, sores, wounds, diarrhoea, and dysentery.

Burlakoti & Kunwar (2008) made an investigation on herbal medicines of Mahakali watershed area, Nepal. It was found that altogether 64 medicinal plants, species belonging to 59 genera, and 43 families were used by local people. Roots (of 25 species) and leaves (of 18 species) were the most frequently used plant parts. There were 6 species of the Euphorbiaceae family found to be used as a medicinal plant. And the seed, latex, fruit were the common parts used for the treatment.

Li et al. (2009) completed the research on aerial parts of *Euphorbia royleana* and found 10 new ingol lathyrene type diterpenoids and 2 known ingenol derivatives. Techniques used are 2D NMR and X-Ray for structure elucidation. Some compounds also possess anti-angiogenic activities.

Sabeen & Ahmad (2009) did research work on the folk medicinal flora of Abbotabad city, Pakistan. They stated that the whole plant of *Euphorbia royleana* is used for different treatments. The stem is used for the treatment of earache and bladder stones. Also, the plant extract with salt is used for the treatment of loose motions.

Xu et al. (2009) state that tirucallane, euphane, triterpenoids including five hydroperoxides, together with 18 known compounds, have been isolated from an ethanolic extract of the roots of *Euphorbia micractina* showed selective cytotoxic

activity against A2780 ovarian cells. M and inhibitory activity against protein tyrosine phosphatase and potent activity against HIV-1 replication.

According to Chanda & Baravalia (2010) the methanol extracts of *E. hirta* and *E. tirucalli* L. exhibit similar activity against a broad spectrum of bacteria and fungi associated with skin infections. The former *E. hirta* plant shows the best antioxidant activity against fungi and gram-positive bacteria, and the later *E. tirucalli* plant shows the best anti-microbial activity. These results support the use of these species in traditional Indian medicine, and they can be used as an easily accessible source of natural antimicrobial agents.

Semwal et al. (2010) reviewed the medicinal plants used by local vaidyas of Ukhimath village, Uttrakhand. Total 60 medicinal plants were studied, and known to cure or used in the treatment of 34 diseases and ailments like headache, intestinal problems. Medicinal plant part latex of *Euphorbia royleana* is also mentioned in the review paper which is locally known as sullu in Uttrakhand, use as an eardrop during secretion of ears.

Mwine & Damme (2011) reviewed that the family Euphorbiaceae is a good topic for research for phytomedicines of veterinary, human, or pesticide nature.

Wang et al. (2011), worked on the evaluation of the cytotoxic activities of extracts of *Euphorbia* genus plants, *Euphorbia fischeriana, Euphorbia tirucalli, Euphorbia humifusa, and Euphorbia antiquorum*, and found that the $CHCl_3$ extracts from *E. tirucalli and E. antiquorum* exhibited a strong cytotoxic effect on the two cell lines. Also, $CHCl_3$ and EtOAc extracts from *E. fischeriana* showed significant cytotoxic activity on the A-549 cell. This study revealed that the CHCl3 extracts from *E. tirucalli and E. antiquorum*, the $CHCl_3$ and EtOAc.

Kumar & Choyal (2012) worked on Traditional Phytotherapy for Snake Bites by the Local Rural People of Hamirpur District in Himachal Pradesh (India). According to the investigation, it reveals that local health healers/rural people used 16 plants belonging to 14 families for the treatment of snake bites. The root of the medicinal plant *Euphorbia royleana* is also used for the treatment of snakebite in the form of decoction with black pepper (kali mirch).

Singh & Singh (2012) worked on the piscicidal and muricidal (mice killing effect) activities of latex extract of *Euphorbia royleana* against freshwater snakehead fish, albino mice Mus musculus and Channa punctatus, and Swiss. The toxicity of latex extract is studied against freshwater fish, Channa punctatus, and was both time and dose-

dependent. But, in the case of mice Mus musculus, the toxicity was only dose-dependent and it was higher in comparison to fish. Therefore, this latex should be used at a lower dose level only for therapeutic purposes and also in an infrequent manner to maintain a healthy environment.

Prakash & Gupta (2013) conclude that the leaves of *Jatropha curcas*, the stem of *Ricinus communis*, and the whole plant of *Euphorbia helioscopia* show highly potent activities even at low concentration (100µg/ml) and suggest that these compounds could be developed further as anticancer drugs.

Tao et al. (2013) conclude that the casbane diterpenes are isolated from the roots of *E. pekinensis*, including four previously unreported compounds. All of the compounds showed cytotoxicity against the human gastric cancer MGC-803, colon cancer SW620, liver cancer SMMC-7721, kidney cancer Ketr-3, breast cancer MCF-7, leukemia cancer HL-60, and lung cancer A549. The C-5 hydroxy group and the double bond played important roles in the structure-activity relationships.

Wang et al. (2011) shows the work on Anti-inflammatory Diterpenoids from the Roots of *Euphorbia ebracteolata* and studied that the three compounds isolated from the roots of *Euphorbia ebracteolata* exhibited significant inhibition of nitric oxide production in RAW 264.7 lipopolysaccharide-induced macrophages, with IC50 values of 2.44, 2.76, and 1.02 µm, respectively, and two new nor diterpene lactones, thirteen diterpenoids, and eight new Rosanne diterpenoids were isolated.

Ashraf et al. (2014) from Pakistan studied antioxidant, antimicrobial, antitumor, and cytotoxic activities of an important medicinal plant *(Euphorbia royleana)* and concluded that hexane extracts have the potential for isolation of antioxidant and antimicrobial compounds for the pharmaceutical industry. It also exhibits antifungal activity against *Aspergillus niger* and antibacterial activity against the gram-positive bacteria *Bacillus subtilis*. As hexane extract also shows potent activity, it was targeted for cytotoxic assessment.

Kichu et al. (2015) from Nagaland worked on the ethnobotanical study of medicinal plants of Chungtia village. A total of 135 plant species of 69 families and 123 genera were recorded in the investigation. The most-reported uses were for gastrointestinal problems, followed by dermatological problems. Plant parts used were leaves, fruits and stems in the form of paste, decoction, infusion, juice or poultice, or taken orally with no

preparation. Medicinal plant *Euphorbia royleana* locally known as Takterak in Nagaland used for the treatment of skin diseases and body pain.

Pandey & Singh (2016) made a survey on "traditional phytotherapy for various diseases by the local rural people of Bharai village in the Kullu district of Himachal Pradesh (India)". Local people still rely on traditional plants for their health care. Survey conducts through personal interviews and questionnaires by local and rural people. Plant *Euphorbia royleana* which is locally known as chuuein in Bharai village used as a healer on cuts and wounds. The root is the plant part that is used in the tradition.

Pisano et al. (2016) worked on significant research on the antibacterial activity of aqueous and ethanolic extracts from *E. characias* leaves, stems, and flowers. Except for the ethanolic extract from leaves against gram-positive bacteria Bacillus subtilis (MIC = 312.5 g/mL), and else results showed that most of these extracts are non-active against the organisms tested (MIC > 1.25 mg/mL.

Perera, Jayawardena & Jayasinghe (2018) well demonstrated the lyophilized decoction of fresh whole *E. hirta* had great potential against the dengue virus since, after 14 days of administration at 100 mg/kg dose, it significantly increased platelet counts in rats, also causing a decrease in coagulation time and bleeding and holds significantly antiviral activities.

Chaitanya & Suresh (2018) explained the uses of *Euphorbia royleana* in the review paper "the neglected anticancer phytoceutical treasures from the Nilgiris biosphere: a short review". *Euphorbia royleana* is a folk medicine in India. It is used as a hair tonic and its latex shows the property of purgative and anthelmintic. Most importantly, in the Ayurveda, the plant is well known for the treatment of male impotency as the latex is similar to goumutra shilajit.

According to Kumar (2018) in ethnobotanical use of some xerophytic plants growing in the dry habitat of Hamirpur district of Himachal Pradesh. *Euphorbia royleana* is a medicinal plant used to treat many ailments. The plant part (crushed stem) is considered antiseptic. Pith slices mixed with wheat dough fed to the cattle to avoid absorption after conception and for stimulating ovulation.

The study by Safwat et al. (2018) was made on the 11 plants of Sahara to discover a Novel anti-tuberculosis agent. Activity determined by the agar diffusion and broth microdilution methods. Methanol extract of *Euphorbia paralias* whole plant was most active against Mycobacterium spp., quercetin-3-O-β-D-glucoside, a safe compound

analyzed by Ultra-violet spectroscopy and Nuclear magnetic resonance is confirmed by an in vitro glutamine synthetase biosynthetic assay that is a promising target for the development of the anti-tuberculosis drug.

Valadao et al. (2018) examined that ethanolic extract of latex from *E. umbellata* has been evaluated for its anti-HIV properties. The study demonstrated in several models (in resting CD4+T cells, in cells from HIV-1 + individuals, and in primary non-human primate CD4+T cells) that this extract can reactivate the HIV in latency, acting on three factors with a synergistic effect.

Silva et al. (2019) state that tetracyclic triterpene alcohol euphol when tested against Jurkat, HL-60, K-562 B16F10, and HRT-18 cell lines, euphol is toxic mainly against K-562 cell line (34.44 μm) by apoptosis induction, with non-toxicity to blood cells (SI > 2), but low selectivity to 3T3 cell line (SI = 0.55). On the other hand, euphol is particularly active against glioma cells (including primary, pediatric, and adult glioma cell lines), with $IC_{50}$ values ranging from 5.98 to 31.05 μm, much more active than the temozolomide, a clinical drug used to treat some brain cancers ($IC_{50}$ values range from 97.00 μm to 1 mm) and with higher selective cytotoxicity index (0.64–3.36) than temozolomide (0.11–1.13).

According to Subramaniyan et al. (2019), herbal medicines are used to treat many ailments if used in medical dosage, as plants are the rich source of bioactive compounds, which are good for humans and also helpful. And they stated that the plant *E. royleana* persists the tumor inhibition based and dose-dependent which is proved through the potato-disc assay.

Wang et al. (2019) isolated the bioactive diterpenoids from the methanolic extracts of the stem of *Euphorbia royleana*. The structure of compounds is determined by the extensive spectroscopic method and ECD data analysis. Lipopolysaccharide stimulated BV-2 cells are used biologically with the 6 diterpenoid compounds for examining nitric oxide inhibitory activity.

Li et al. (2020) researched Cytotoxic Lathyrane Diterpenoids from the Roots of *Euphorbia fischeriana*. Compound isolation was done by the extraction and partitioned method in 95% ethyl alcohol followed by column chromatography and HPLC (high-performance liquid chromatography).

Shaker et al. (2020) isolates the 22 known analogues and also 3 ingenanes, 2 membranes, 1 ent-kaurane, and 2 ent-atisanes from *Euphorbia royleana* whole plant, and all the

isolated compounds screened for their chemoreversal abilities on p-glycoprotein mediated multi-drug cancer cell line HepG2/DOX. Structure elucidation was done by spectroscopic analysis, single-crystal X-ray diffraction, and chemical transformation. Ingol-3,7,12-triacetate-8-benzoate was found to be the most activated MDR modulator and could enhance the efficacy of the anti-cancer drug.

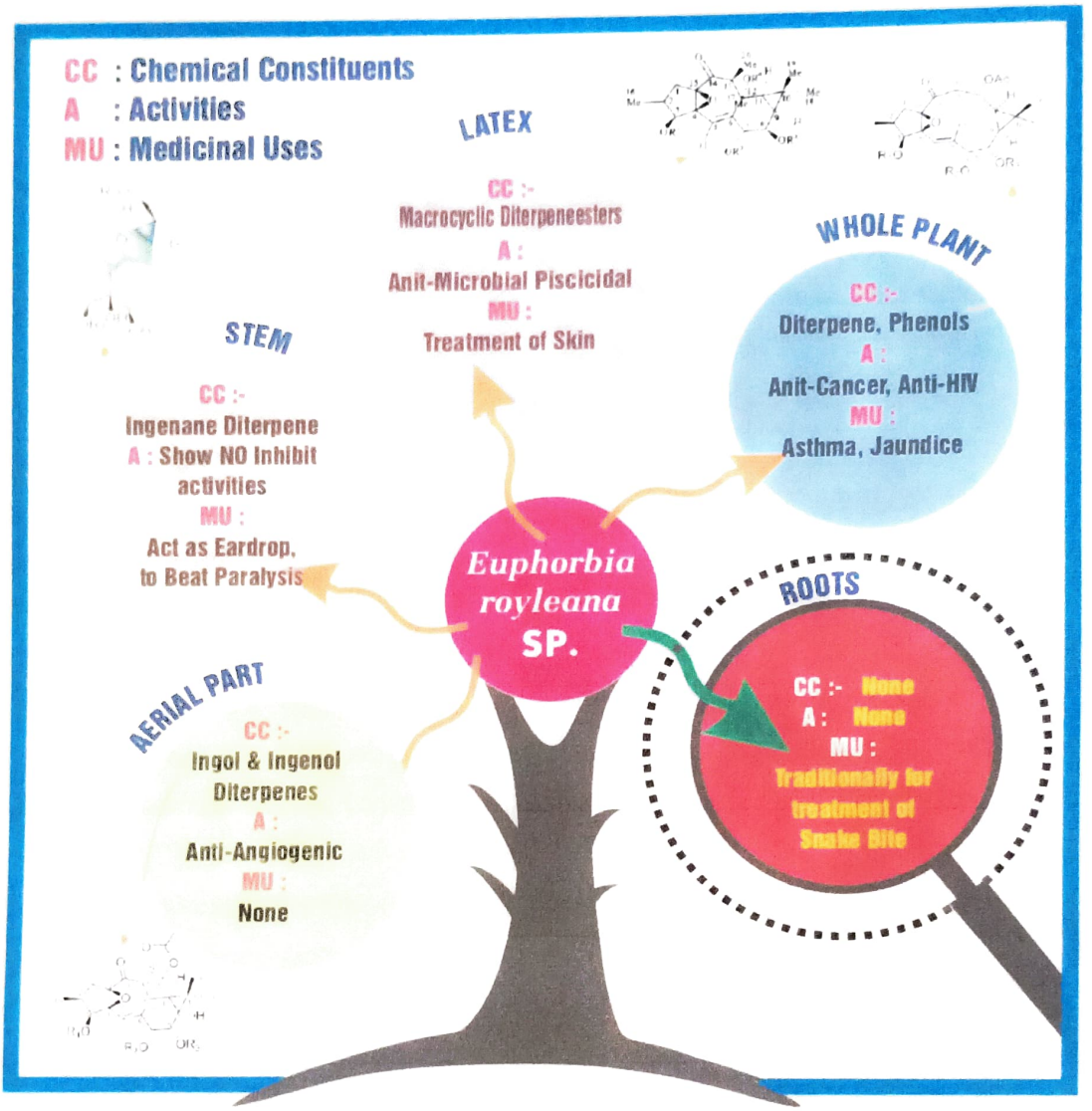
Kemboi, Peter, Langat & Tembu (2020) made a review on ethnomedicinal use, biological activities, triterpenoids of *Euphorbia* species. The most-reported triterpenoids in the review are oleanane, lupane, ursane, tirucallane, cycloartanes, and taraxane subcla. So, the report is made on the triterpenoids with interesting anticancer activities that may help in clinical trials with regards to drug discovery. Plant *Euphorbia royleana* is also mentioned in the report and the whole plant is used for the treatment of jaundice, asthma, and cough. Plants show antibacterial and anti-HIV biological properties.

## Research Gap Identified

India is rich in flora and fauna and thus rich in the availability of medicinal plants. Extracts of many plants used in the treatment of disease in the medicine system of Ayurveda. The Literature reveals that various studies have been conducted on the family Euphorbiaceae and they have been used to treat analgesia, inflammation, jaundice, etc., and also focused on antifungal, anti-inflammatory & antidiuretic activity. Species of the *Euphorbia* genus show cytotoxic, antitumor or, anti-venom activities. So, on the Despite of exhaustive work up till now on genus euphorbia (plant parts used are stem, latex, leaves). There is no systematic study carried out on identification, isolation, characterization, and structure elucidation of active principles from the root extract of *Euphorbia royleana.*

Genus *Euphorbia* has a strong medicinal background so is selected for further investigation, and there is a complete research gap of knowledge about the medicinal attributes of roots of *Euphorbia royleana.* Therefore, the present work focused on the chemical profiling of different parts of plant. Also, isolation, characterization, and structure elucidation of active principles of plant parts for biological properties.

## GRAPHICAL RESEARCH GAP




### Objectives:

The following objectives are designed to fulfil the aim.

An attempt has been made to study identification, isolation, characterization, and structure elucidation of bioactive compounds, followed by the shade dry of plant parts and extraction with different solvents.

The main parts of the study are:

- Extraction, fractionation and isolation of compounds of *Euphorbia royleana.*
- Metabolomics study of *Euphorbia royleana* parts.
- In vitro screening of extracts, fractions and pure molecule for their therapeutic potential.

## Proposed Methodology

The following step will be taken during the research work are given below:

**(1) Study area:** Plant *Euphorbia royleana* commonly occurs in dry hills tracts of outer Himalayas up to 2000 m from Kumaon to Jhelum (Mehra, P.N. & Choda, S.P. 1978), the north-western part of India. The places of Himachal Pradesh where *Euphorbia royleana* is found are Hamirpur, Mandi, Chamba, Kullu, Una, Kangra, Bilaspur. A study will be carried out in the sub mountains, low hills subtropical zone areas (district Hamirpur, district Bilaspur and district Mandi). Samples will be collected from the village Tikker (latitude: 31° 37' 44.5512" & longitude: 76° 37' 36.1128"), Pungh (latitude: 31° 31' 13.9872" & longitude: 76° 52' 41.8152") and Bagharin (latitude: 31° 24' 10.602" & longitude: 76° 47' 34.3284") of district Hamirpur, Mandi and Bilaspur respectively.

**(2) Sampling of plant material:** The parts of *Euphorbia royleana* will be collected from the 3 different districts of Himachal Pradesh. The different parts of the collected plant will be separated manually, washed with fresh tap water 4-5 times, and finally with distilled water to ensure that plant parts are free from any foreign material or dust sticking to the parts during the investigation. The mature, disease-free and clean parts will be dried in shade. Then after that dried parts of *Euphorbia royleana* would be powdered, sieved and fine powder then obtained be packed and kept for further investigation.

**(3) Preparation of plant extract**: The preparation of the extract of medicinal plant done with the help of the process of maceration. In this process, the fine powder is to be placed in the stoppered container with mixed solvent. After the mixing of solvent, the mixture is allowed to stand for at least 3-4 days at room temperature till the solvent constituents dissolve into it. The next step is to add the stain to the mixture. Now combined liquids are elucidated by filtration or decantation after standing. The entire step should be taken carefully to assure that active constituents are not lost, destroy and distort during the preparation of extract (Sasidharan et al., 2011)

**(4) Isolation of compounds**: The isolation of compounds from parts of *Euphorbia royleana* is to be done with help of various chromatographic techniques such as TLC, LCMS, and column chromatography (Sasidharan et al., 2011)

**(5) Chemical profiling:** Chemical profiling of different parts of *Euphorbia royleana* using advanced analytical techniques (LC-MS, MS, HPTLC &NMR)

**(5) Characterization and structural elucidation of active principles**: The characterization and structural elucidation of active principles are to be done by the application of some spectroscopic techniques like UV, IR, and NMR spectroscopy (Sasidharan et al., 2011).

**(6) Biological Activity**: The biological activity deals with the different activities such as antibacterial, antifungal, anti-inflammatory, antimicrobial, antioxidant, anticancer antitumor activities, and those activities are determined by using a standard protocol.

## Thesis Outline

**Chapter 1: - (Introduction)** The chapter will include the introduction of natural products and genus *Euphorbia*, literature review, reasons for selecting the plant. The chapter will also include about the biological activities.

**Chapter 2: - (Literature Review)** This chapter include the various hypothesis and research of different authors. This chapter also includes the research gap identified.

**Chapter 3: - (Materials and Methods)** This chapter includes the details of the experimental used and instrumental details. This chapter also includes the techniques and steps used in the research.

**Chapter 4: - (Results and Discussions)** This chapter deals with the results/outcomes of the identification and solation of the compound. It also includes about the results of bioactivity of different isolated compounds.

**Chapter 5: - (Summary and Conclusions)** This chapter will comprise summary of entire study according to objectives along with conclusions. This chapter also include the future scope of the research.

**Chapter 6: - (References)** This chapter includes the references of books, reports, research papers of the authors associating in the ongoing research.

## References

Ahmed, J., Preissner, S., Dunkel, M., Worth, C.L., Eckert, A. & Preissner, R. (2011) Supersweet—a resource on natural and artificial sweetening agents. *Nucleic Acids Research*. 39(1), D377-D382. Available from: https://doi.org/10.1093/nar/gkq917 [Accessed 22nd Dec. 2021]

Andrae-Marobela, K., Ghislain, F.W., Okatch, H. & Majinda, R.R.T. (2013) Polyphenols: A diverse class of multi-target anti-HIV agents. *Current Drug Metabolism*. 14(4), 392-413. Available from: https://pubmed.ncbi.nlm.nih.gov/23330927/ [Accessed 13th Nov. 2021]

Ashraf, A., Sarfraz., R.A., Rashid, M.A. & Shahid, M. (2014) Antioxidant, antimicrobial, antitumor, cytotoxic activities of an important medicinal plant (Euphorbia royleana) from Pakistan. *Journal of food and drug analysis.* 23(1), 1-7. Available from: https://doi.org/10.1016/j.jfda.2014.05.007 [Accessed 13th Dec. 2021]

Atanasov, A.G., Waltenberger, B., Pferschy, E.M., Linder, T., Wawrosch, C., Uhirin,P., Temm, V., Wang, L., Schwaiger. S., Heiss, E.H., Rollinger, J.M., Schuster, D, Breuss, J.M., Bochkoy, V., Milhovilovic, M.D., Kopp, B., Bauer, R., Dirsch, V.M., Stuppner, H. (2015) Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances.* 33(8), 1583-1585. Available from: https://doi.org/10.1016/j.biotechadv.2015.08.001[Accessed 24th Oct. 2021]

Bani, S., Kaul, A., Jaggi, B.S., Suri, K.A., Suri, O.P. & Sharma, O.P. (2000) Anti-inflammatory activity of the hydrosoluble fraction of Euphorbia royleana latex. *Fitoterapia.* 71(6), 655-662. Available from: https://doi.org/10.1016/S0367-326X(00)00225-2 [Accessed 13th Dec. 2021]

Bani, S., Kaul, S., Khan, B., Ahmad, S.F., Suri, K.A., Satti, N.K., Amina, M., & Qazi, G.N. (2005) Immunosuppressive properties of an ethyl acetate fraction from Euphorbia royleana. *Journal of Ethnopharmacology.* 99 (2), 185–192. Available from: DOI: 10.1016/J.JEP.2004.12.2017 [Accessed 20th Dec. 2021]

Boots, A.W., Haenen, G.R. & Bast, A. (2008) Health effects of quercetin: from antioxidant to nutraceutical. *European Journal Of Pharmacology.* 585(2-3), 325-337. Available from: http://dx.doi.org/10.1016/j.ejphar.2008.03.008 [Accessed 21st Jan. 2022]

Burlakoti, C. & Kunwar, R.M. (2008) Folk herbal of Mahakali watershed, Nepal. *Medicinal plants in Nepal: An Anthology Of Contemporary Research.* 187-189. Available from: https://www.researchgate.net/publication/257308463 [Accessed 20th Nov. 2021]

Chaitanya, M.V.N.L. & Suresh, P. (2018) The neglected anticancer phytoceutical treasures from the Nilgiris biosphere: A short review. *Journal Of Pharmaceutical Research International.* 22(1), 3. Available from: https://www.journaljpri.com/index.php/JPRI/article/view/19118 [Accessed 15th Nov. 2021]

Chanda, S. & Baravalia, Y. (2010) Screening of some plant extracts against some skin diseases caused by oxidative stress and micro-organisms. *African Journal Of*

*Biotechnology*. 9(21), 3210-3217. Available from: http://www.academicjournals.org/AJB [Accessed 24th Nov. 2021]

Dafni, A. (2006) On the topology and the worship status of sacred trees with a special reference to the middle east. *Journal Of Ethnobiology And Ethnomedicine*. 2(26), 1-14. Available from: https://doi.org/10.1186/1746-4269-2-26 [Accessed 12th Dec. 2021]

Dogra, K.S., Kohil, R.K., Sood, S.K. & Dobhal, P.K. (2009) Impact *of Ageratum conyzoids* L. on the diversity and composition of vegetation in the Shivalik hills of Himachal Pradesh (Northwestern Himalaya), India. *Internation Journal Of Biodiversity And conservation*. 14, 135-145. Available from: http://repository.ias.ac.in/76500/1/2-p.pdf [Accessed 12th Dec. 2021]

Dunkel, M., Schmidt,U, Struck, S., Berger, L., Gruening, B., Hossbasch, J., Jaegar, I.S., Effmert, U., Piechulla,B., Eriksson, R., Knudsen, J. & Preissner, R. (2009) SuperScent-a database of flavors and scents, *Nucleic-Acids Research*. 37(1), D291-D294. Available from: https://doi.org/10.1093/nar/gk695 [Accessed 18th Nov. 2021]

Ernst, M., Grace, O.M., Saslis-Lagoudakis, C.H., Nilsson, N., Simonsen, H.T., Ronsted, N. (2015) Global medicinal uses of Euphorbia L. (Euphorbiaceae). *Journal Of Ethnopharmocology*. 176, 14-15. Available from: https://doi.org/10.1016/j.jep.2015.10.025 [Accessed 13th Dec. 2021]

Gurib-Fakim, A. (2006) Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects Of Medicine*. 27(1), 3. Available from: https://doi.org/10.1016/j.mam.2005.07008 [Accessed 20th Dec. 2021]

Harvey, A.L., Edrada-Ebel, R. & Quinn, R.J. (2015) The re-emergence of natural products for drug discovery in the genomics era. *Natural Reviews Drug Discovery*. 14(2), 1-2. Available from: https://doi.org/10.1038/nrd4510 [Acceessed 24th Oct. 2021]

Kargıoglu, M., Cenkci, S., Serteser. A., Evliyaoglu N., Konuk, M., Kök, M.S. & Bagcı, Y. (2008) An ethnobotanical survey of Inner-West Anatolia. *Human Ecology*. 36(1), 763. Available from: https://doi.org/10.1007/s10745-008-9198-x [Accessed 12th Dec. 2021]

Kemboi, D., Peter, X., Langat, M., Tembu, J. (2020) A review of the ethnomedicinal uses, biological activities, and triterpenoids of Euphorbia species. *Molecules*. 25(17), 1-29. Available from: https://www.mdpi.com/1420-309/25/17/4019 [Accessed 13th Dec. 2021]

Kichu, M., Malewska, T., Akter, K., Imchen, J., Harrington, D., Kohen, J., Vemulpad, S.R. & Jamie, J.F. (2015) An ethnobotanical study of medicinal plants of Chungtia village, Nagaland, India. *Journal Of Ethnopharmacology.* 166, 5-17. Available from: https://doi.org/10.1016/j.jep.2015.02.053 [Accessed 21st Nov. 2021]

Kumar, S., Malhotra, R. & Kumar, D. (2010) *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacognosy Review.* 4(7), 58. Available from: https://www.researchgate.net/publication/221732777 [Accessed 12th Dec. 2021]

Kumar, N. & Choyal R. (2012) Traditional phytotherapy for snake bites by local rural people of Hamirpur District in Himachal Pradesh (India) *Biological Forum- An International Journal.* 4(1), 98-106. Available from: https://www.researchtrend.net/bfij/biological_forum_4(1)_2012/19%20NITESH%20HP.pdf [Accessed 14th Dec. 2021]

Li, J., He, J., Yang, C., Yan, X., & Zhao, Y. (2020) Cytotoxic Lathyrane Diterpenoids from the Roots of Euphorbia fischeriana. *Records of natural products.* 14(4), 3. Available from: https://doi.org/10.25135/rnp.167.19.12.1496 [Accesssed 21st Nov. 21]

Li, X.L., Li, Y., Wang, S.F., Zhao, Y.L., Liu, K.C., Wang, X.M. & Yang, Y.P. (2009) Ingol and ingenol diterpenes from the aerial parts of Euphorbia royleana and their antiangiogenic activities. *Journal Of Natural Products.* 72(6), 1001-1005. Available from: https://doi.org/10.1021/np800816n [Accessed 15th Nov. 2021]

Liu, Z.G., Li, Z.L., Bai, J., Meng, D.L., Li, N., Pei, Y.H., Feng, Z. & Hua, H.M. (2014) Anti-inflammatory diterpenoids from the roots of Euphorbia ebracteolate. *Journal of natural products.* 77, 792-778. Available from: https://doi.org/10.1021/np400873v [Accessed 20th Dec. 2021]

Mehra, P.N. & Choda, S.P. (1978) cyto-taxonomical studies in the genus Euphorbia L. *Cytologia.* 43(2), 217-235. Available from: https://doi.org/10.1508/cytologia.43.217 [Accessed 21st Nov. 2021]

Mustafa, G., Arif, R., Atta, A., Sharif, S. & Jamil, A. (2017) Bioactive compounds from medicinal plants and their importance in drug discovery in Pakistan. *Matrix Science Pharma.* 1(1), 17-26. Available from: https://www.researchgate.net/publication/315832271. [Accessed 13th Dec. 2021]

Mwine, J.T. & Damme, P.V. (2011) Why do Euphorbiaceae tick as medicinal plants? A review of the Euphorbiaceae family and its medicinal features. *Journal Of Medicinal*

*Plants Research.* 5(5), 652. Available from: http://www.academicjournals.org/JMPR [Accessed 13th Dec. 2021]

Nadkarni, K.M. & Nadkarni,A.K. (1982) *The Indian materia medica.* Edition 3. London. Popular Book Depot. Available from: https://books.google.com/books/about/Dr_K_M_Nadkarni_s_Indian_Materia_Medica.html?id=sH0sSAAACAAJ [Accessed 13th Dec. 2021]

Newman, D. J. & Cragg, G. M. (2020) Natural products as sources of new drugs from 01/1981 to 09/2019. *Journal Of Natural Products.* 83(3), 770-803. Available from: https://pubs.acs.org/doi/10.1021/acs.jnatprod.5b01055 [Accessed 13th Dec. 2021]

Ogbulie, J.N., Ogueke, C.C., Okoli, C. & Anyanwu, B.N. (2007) Antibacterial activities and toxicology potentials of crude ethanolic extracts of Euphorbia hirta. *African Journal Of Biotechnology.* 6(13), 1544-1548. Available from: http://www.academicjournals.org/AJB [Accessed 10th Nov. 2021]

Pandey, A. & Singh, S. (2016) Traditional phytotherapy for various diseases by the local rural people of Bharai village in the Kullu district of Himachal Pradesh. *International Journal Of Pharmaceutical Sciences And Research.* 7(3), 1263-1270. Available from: https://ijpsr.com/bft-article/traditional-phytotherapy-for-various-diseases-by-the-local-rural-people-of-bharai-village-in-the-kullu-district-of-himachal-pradesh-india/ [Accessed 15th Nov. 2021]

Pascal, A.O., Bertrand, A.E.V., Esaie, T., Sylvie, H.A.M., & Eloi, A.Y. (2017) A review of the ethnomedical uses, phytochemistry, and pharmacology of the Euphorbia genus. *The Pharma Innovation Journal.* 6(1), 34-39. Available from: https://www.researchgate.net/publication/340024139_A_review_of_the_ethnomedical_use_phytochemistry_and_pharmacology_of_the_Euphorbia_genus [Accesssed 18th Dec. 2021]

Paudel, M.R., Paudel, B., Bhattarai, H.D., Kunwar, R.M., Jan, H.A., Turi, M.A., Bussmann, R.W. & Paniagua-Zambrana, N.Y. (2021) *Euphorbia hirta* L., *Euphorbia Pilosa* L., *Euphorbia royleana* Boiss. *Ethnobotany of the Himalayas.* 1-14. Available from: 10.1007/978-3-030-45597-2_97-2 [Accessed 10th Dec. 2021]

Perera, S.D., Jayawardena, U.A. Jayasinghe, C.D. (2018) Potential use of Euphorbia hirta for dengue: A systematic review of scientific evidence. *Journal of Tropical Medicine.* 2018, 1-6. Available from: https://doi.org/10.1155/2018/2048530 [Accessed 11th Nov. 2021]

Pisano, M.B., Cosentino, S., Viale, D., Spano, D., Corona, A., Esposito, F., Tramontano, E., Montoro, P., Tuberoso, I.G., Medda, R. & Pintus, F. (2016) Biological activities of aerial parts extracts of Euphorbia characias. *BioMed Research International*. 2016, 1-11 Available from: https://doi.org/10.1155/2016/1538703 [Accessed 10th Nov. 2021]

Prakash, E. & Gupta, D.K. (2013) Cytotoxic activities of extracts of medicinal plants of Euphorbiaceae family studied on seven human cancer cell lines. *Universal Journal Of Plant Science*. 1(4), 113-117. Available from: http://www.hrpub.org/journals/article_info.php?aid=824 [Accessed 13th Dec. 2021]

Rizk, A.M., Hammouda, F.M., El-Missiry, M.M., Radwan, H.M. & Evans, F.J. (1984) Macrocyclic diterpene esters from euphorbia royleana. *Phytochemistry*. 23(10), 2377-2379. Available from: DOI: 10.1016/s0031-9422(00)80559-2 [Accessed 10th Nov. 2021]

Rizk, A.M. (1987) The chemical constituents and economic plants of the Euphorbiaceae. *Botanical Journal Of Linenean Society*. 94(1-2), 294-396. Available from: https://doi.org/10.1111/j.1095-8339.1987.tb01052.x. [Accessed 15th Nov. 2021]

Robards, K., Prenzler, P.D., Tucker,G., Swatsitang, P. & Glover,W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry*. 66(4), 401-436. Available from: https://doi.org/10.1016/S0308-8146(99)00093-X [Acessed 24th Nov. 2021]

Sabeen, M. & Ahmad, S.S. (2009) Exploring the folk medicinal flora of Abbotabad city, Pakistan. *Ethnobotanical Leaflets*. 18, 810-833. Available from: https://opensiuc.lib.siu.edu/ebl/vol2009/iss7/1 [Accessed 20th Nov. 2021]

Safwat, N.A., Kashef, M.T., Aziz, R.K., Amer, K.F., Ramadan, M.A. (2018) Quercetin 3-O-glucoside recovered from the wild Egyptian Sahara plant, Euphorbia paralias L., inhibits glutamine synthetase and has antimycobacterial activity. *Tuberculosis*. 108, 106–113. Available from: http://doi.org/10.1016/j.tube.2017.11.005 [Accessed 10th Nov. 2021]

Salehi, B., Iriti, M., Vitalini, S., Antolak, H., Pawlikowska, E., kregiel, D., Sharifi-Rad, J., Oyeleye, S.I., Ademiluyi, A.O., Czopek, K., Staniak, M., Custodio, L., Coy-Barrera, E., Segura-Carretero, A., Cádiz-Gurrea, M.D.L.L., Capasso, R., Cho, W.C., & Seca, A.M.L. (2019) Euphorbia- derived natural products with potential for use in health maintenance. Euphorbia-Derived Natural Products with Potential for Use in Health Maintenance. *Biomolecules*. 9(8), 1-2. Available from: https://doi.org/10.3390/biom9080337 [Accessed 10th Nov. 2021]

Sasidharan, S, Chen, Y, Saravanan, D., Sundram, K. & Yoga, L. (2011) Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal Of Traditional, Complementary, And Alternative Medicines.* 8(1), 1-10. Available from: http://pubmed.ncbi.nlm.nih.gov/22238476/ [Accessed 13th Dec. 2021]

Seigler, D.S., (1994) Phytochemistry and Systematics of the Euphorbiaceae. *Annals of the Missouri Botanical Garden.* 81(2), 380-381. Available from: https://www.biodiversity.org/part/30312 [Accessed 11th Nov. 2021]

Semwal, D.P., Saradhi, P.P., Kala, C.P. & Sajwan, B.S. (2010) Medicinal plants used by vaidyas in Ukimath block, Uttrakhand. *Indian Journal Of Traditional Knowledge.* 9(3), 480-485. Available from: https://www.researchgate.net/publication/265844778_Medicinal_plants_used_by_local_Vaidyas_in_Ukhimath_block_Uttarakhand_India [Accessed 21st Nov. 2021]

Shakera, S., Sanga, J., Yana, X.L., Fana, R., Tanga, G., Xub, Y. & Yina, S. (2020) Diterpenoids from Euphorbia royleana reverse P-glycoprotein-mediated multidrug resistance in cancer cells. *Phytochemistry.* 176, 112395. Available from: https://doi.org/10.1016/j.phytochem.2020.112395 [Accessed 30th Nov. 2021]

Shi, Q.W., Su, X.H. & Kiyota, H. (2008) Chemical and Pharmacological Research of the Plants in Genus Euphorbia. *Chemical Reviews.* 108(10), 4295-4327. Available from: https://doi.org/10.1021/cr078350s [Accesssed 10th Nov. 2021]

Singh, P. & Singh, A. (2012) Evaluation of latex of extract of Euphorbia royleana for its piscicidal and muricidal activities. *World Journal Of Agricultural Sciences.* 8(5), 520-524. Available from: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.415.2553&rep=rep1&type=pdf [Accessed 13th Dec. 2021]

Sorokina, M. & Steinbeck, C. (2020) Review on natural products databases: where to find data in 2020. *Journal of Cheminform.* 12(20),1-2. Available from: https://doi.org/10.1186/s13321-020-00424-9 [Accessed 25th Oct. 2021]

Sparks, T.C., Wessels, F.J., Lorbasch, B.A., Nugent, B.M., Watson, G.B. (2019) The new age of insecticide discovery- the crop protection industry and the impact of natural products. *Pesticide Biochemistry and Physiology.* 161, 12-22. Available from: https://doi.org/10.1016/j.pestbp.2019.09.002 [Accessed 17th Dec. 2021]

Subramaniyan, V., Kayarohanam, S., Kumar, A.J. & Kumarsamy, V. (2019) Impact of herbal drugs and its clinical applications. *International Journal Of Research In*

*Pharmaceutical Sciences.* 10(2), 1340-1345 Available from: https://doi.org/10.26452/ijrps.v10i2.537 [Accessed 10th Oct. 2021]

Suffredini, I.B., Sader, H.S., Goncalves, A.G., Reis, A.O. Gales, A.C., Varella, A.D. & Younes, R.N. (2004) Screening of antibacterial extracts from plants native to the Brazilian Amazon rain forest and Atlantic rain forest. *Brazilian Journal Of Medical And Biological Research.* 37(3), 379-384. Available from: http://dx.doi.org/10.1590/S0100-879X2004000300015 [Accessed 12th Jan. 2022]

Tao, W.W., Duan, J.A., Tang, Y.P., Yang, N.N., Li, J.P. & Qian, Y.F. (2013) Casbane diterpenoids from the roots of Euphorbia pekinensis. *Phytochemistry.* 94, 249-253. Available from: http://dx.doi.org/10.1016/j.phytochem.2013.06.009 [Accessed 20th Dec. 2021]

Thakur, S. & Sood, S.K. (2001) *Studies on the ethnobotany of Rewalsar_distt. Mandi Himachal Pradesh, India.* Ph.D. thesis. Himachal Pradesh University, Shimla. Available from: http://hdl.handle.net/10603/122061 [Accessed 13th Nov. 2021]

Tiwari, S., & Singh, A.V. (2004) Piscicidal and anti-acetylcholinesterase activity of Euphorbia royleana stem bark extracts against freshwater common predatory fish Channa punctatus. *Environmental toxicology and pharmacology.* 181, 47-53. Available from: https://doi.org/10.1016/j.etap.2004.05.001 [Accessed 13th Nov. 2021]

Valadao, A.L.C., Pezzuto, P., Silva, V.A.O., Goncalves, B.S., Rossi, A.D., da Cunha, R.D., Siani, A.C., Tostes, J.B.F., Trovo. M., Damasco. P., Goncalves, G., Reis, R.M., Aguiar, R.S., Bento, C.A.M., Tanuri, A. (2018) Reactivation of latent HIV-1 in vitro using an ethanolic extract from Euphorbia umbellata (Euphorbiaceae) latex. *PLOS One.* 13(11). Available from: https://doi.org/10.1371/journal.pone.0207664 [Accessed 12th Dec 2021]

Vasas, A. & Hohmann J. (2014) Euphorbia Diterpenes: Isolation, structure, Biological Activity, and synthesis (2008-2012). *Chemical Reviews.* 114(17), 8579. Available from: https://doi.org/10.1021/cr400541 [Accessed 13th Dec. 2021]

Waltenberger, B., Mocan, A., Smejkal, K., Heiss, E.H.E.H. & Atanasov, A.G. (2016) Natural products to counteract the epidemic of cardiovascular and metabolic disorders. *Molecules.* 21(6). Available from: https://www.researchgate.net/publication/304369243 [Accessed 22nd Nov. 2021]

Wang, P., Xie, C., An, L., Yang, X., Xi, Y., Yuan, S., Zhang, C., Tuerhong, M., Jin, D., Lee, D., Zhang, J., Ohizumi, Y., Xu, J. & Guo, Y. (2019) Bioactive diterpenoids from

the stems of Euphorbia royleana. *Journal Of Natural Products.* 82(2), 183-193. Available from: https://doi.org/10.1021/acs.jnatprod.8b00493 [Accessed 12th Dec. 2021]

Wang, X.Y., Liu, L.P., Zhu, R,X., Kang, T.G., Tong, L.J., Xie, H. & Wang, H.B. (2011) Cytotoxic activities of some selected medicinal plants of the genus Euphorbia. *Journal Of Medicinal Plants Research.* 5(31), 6766-6769. Available from: http://www.academicjournals.org/jMPR [Accessed 20th Nov. 2021]

Webster, G.L. (1994) Classification of the Euphorbiaceae. *Annals Of The Missouri Botanical Garden.* 81, 3. Available from: https://doi.org/10.2307/2399908 [Accessed 13th Dec. 2021]

Wu, Z., Raven, P.H., Hong, D. (n.d.) *Flora of China.* Beijing/St. Louis. Science Press/Missouri Botanical Garden Press. 289-300. [Accessed 10th Jan. 2022]

Xiao, J., Muzashvili, T.S. & Georgieve, M.I. (2014) Advances in the biotechnological glycosylation of valuable flavonoids. *Biotechnology Advances.* 32(16),1-2. Available from: https://doi.org/10.106/j.biotechadv.2014.04.006 [Accessed 13th Nov. 2021]

Xu, W., Zhu, C., Cheng, W., Fan, X., Chen, X., Yang, S., Guo, Y., Ye, F. & Shi, J. (2009) Chemical constituents of the roots of Euphorbia micractina. *Journal of natural products.* 72(9), 1620-1626 Available from: https://doi.org/10.1021/np900305j [Accessed 13th Dec. 2021 ]

Yue, Y., Chu, G.X., Liu, X.S., Tang, X., Wang, W., Liu, G.J., Yang, T., Ling, T.J., Wang, X.G., Zhang, Z.Z., Xia, T., Wan, X.C. & Bao, G.H. (2014) TMDB; a literature-curated database for small molecular compounds found from tea. *BMC Plant Biology.* 14, 243. Available from: https://doi.org/10.1186/s12870-014-0243-1 [Accessed 25th Nov. 2021]

Zhang, Q., Zhou, Q.R., Lou, J.W., Chen, P.D., Yao, W., Tao, W.W., Tang, Y.P., Dai, G.C., Wang, K. & Zhang, L. (2017) Chemical Constituents from Euphorbia kansui. *Molecules.* 22(12), 2176. Available from: https://doi.org/10.3390/molecules22122176

Zheng, W.F., Cui, Z., & Zhu, Q.X. (1998) Cytotoxicity and antiviral activity of the compounds from Euphorbia kansui. *Planta Medica.* 64(8), 754-756. Available from: https://doi.org/10.1055/s-2006-957574 [Accessed 13th Nov. 2021]

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# CERTIFICATE
## (SYNOPSIS APPROVAL CERTIFICATE)

This is to certify that the Synopsis titled,' **Identification, Isolation, and Characterization of Active Principals from *Euphorbia royleana*"**, submitted by **Ms. Pallavi Kumari** to the Career Point University, Hamirpur (H.P.), India in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in theDepartment of Chemistry, **School of Basic and Applied Sciences** has been approved by the Research Advisory Committee (RAC) after an Oral Examination of the student on dated 28/02/22.

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